

Methods: Using studies based on individuals with known dates of HIV seroconversion among MSM population from Beijing RRIMO cohort and CASCADE seroconverter cohorts, we compared rates of CD4 decline (on the cube root scale) using linear mixed models. All patients were antiretroviral naïve during follow-up for these analyses.

Results: For 131 Beijing and 3171 CASCADE MSM infected 2001–2008, the rates of decline were estimated to be -0.59 (95%CI $-0.72, -0.47$) and -0.41 ($-0.44, -0.38$) cubic root of CD4 per year, respectively. The CD4 count at time of seroconversion estimated from the model for the Beijing PRIMO group was 504 cells/mm³ (95%CI 463, 547), lower than 554 (95%CI 544, 564) cells/mm³ estimated for the CASCADE group. These rates were equivalent to an estimated average CD4 cell loss in the first two years following HIV seroconversion of 194 and 149 cells/mm³, respectively. Difference in rate of decline on the cubic root scale persisted even after adjusting for CD4 count and age at seroconversion (0.19, 95%CI 0.06–0.32). Subtype data were available for 117 and 1198 Primo and CASCADE MSM, of whom the majority (58.12%, 68/117 and 93%, 1109/1198) were subtypes AE and B, respectively.

Conclusions: These data suggest that CD4 cell loss may be particularly rapid among MSMs in China, compared with that observed in industrialised countries. Further data are required to confirm this observation.

OL-065 Clinical application and study of a HIV-1 diagnosis assay with PCR

L.L. Dai¹, D.X. Chen¹, Y. Shi¹, F.L. Wei¹, T. Zhang^{1*}. ¹Youan hospital, China

Background: To establish the HIV-1 nPCR and RT-PCR diagnosis assay system.

Methods: Establish an nPCR HIV DNA and RT-PCR HIV RNA detection system with three sets of primers which targeting gag, pol and gp41 districts in HIV-1. 119 HIV seropositive patients were tested by PCR and NASBA separately, the sensitivities were compared. The sensitivities of PCR detection in patients with different viral load were compared. 10 cases of suspected acute infection patients were tested by using the established PCR method. The C2-C3 district in HIV-1 membrane was amplified in 43 positive DNA to identify their subtype.

Results: Sensitivity of nPCR is 97.5% (116/119). Sensitivity of RT-PCR is 78.2% (93/119). Specificities of both assay systems are 100% (50/50). The positive predictive values are 97.5% (116/119) and 78.2% (93/119) respectively, and the negative predictive values are both 100% (50/50). The accuracies are 98.2% and 84.6%. The nPCR assay systems showed higher sensitivity than NASBA (97.5% VS. 73.1%, $\chi^2 = 28.17$, $P < 0.01$). RT-PCR has no significant higher sensitivity than NASBA (82.8% VS. 75%, $\chi^2 = 0.821$, $P = 0.365$). The sensitivity of nPCR is markedly higher than RT-PCT in patients with $<10^3$ copy/ml viral load ($P = 0.000$), but the sensitivities of nPCR and RT-PCT are close to each other (nearly 100%) in patients with 10^3 – 10^4 copy/ml viral load ($P = 0.491$) and $\geq 10^4$ copy/ml viral load ($P = 1.000$). 5 of the 10 cases of suspected acute infection patients were tested positive by the PCR, and has a seropositive conversion afterwards in the follow up, therefore confirmed to be HIV acute infection. The 43 DNA samples belong to subtype B' (37 cases), AE subtype (5 cases) and BC subtype (1 case).

Conclusion: The established PCR detective method have a wonderful expansion effect on subtype virus strain B', AE and BC, and get a higher detective sensitivity than NASBA.

OL-066 Cellular factors and HIV-1 reverse transcription

K. Warren¹, T. Wei^{*}, D.S. Li¹, D. Warrilow², D. Harrich¹. ¹HIV Molecular Virology Laboratory, Division of Infectious Diseases, Queensland Institute of Medical Research, Herston, Qld 4029, Australia, ²Public Health Virology Laboratory, Queensland Health Forensic and Scientific Services, PO Box594, Archerfield, Qld 4108, Australia

Introduction: HIV-1 reverse transcription is an essential step of HIV-1 replication during which the viral positive strand RNA genome is converted to double strand DNA. The RNA/DNA dependent polymerase, reverse transcriptase (RT), completes this process using two DNA strand transfer events in addition to typical DNA synthesis. Our previous *in vitro* data supported our initial hypothesis that cell factors must play an important role during viral DNA synthesis. Our endogenous reverse transcription (ERT) assays showed that cellular lysates could enhance viral DNA synthesis up to 30-fold and only late steps of the reverse transcription process were affected. In this study, we identified candidate proteins from cellular lysates by conventional chromatography and mass spectrometry and then investigated their effect on HIV-1 reverse transcription during both *in vitro* ERT assay and cell infections.

Methods: Human cell lysates were added to purified, delipidated virions in an ERT assay and products detected by qPCR. Lysates were purified by conventional chromatography and candidate factors were identified by mass spectrometry. The requirement of candidate factors for the previously described activity was determined by selectively depleting factors from purified 'active' lysate fractions and testing by ERT assay. siRNAs were synthesized targeting candidate proteins that were shown to affect HIV-1 reverse transcription in ERT assay. The effect of targeted cellular proteins on HIV-1 replication was then investigated in cells.

Results: Thirty candidate proteins were consistently identified by mass spectrometry including proteins involved in translation, transcription, RNA metabolism, protein transport, localization and protein folding. Our ERT data showed that cellular translation elongation factors and/or their relevant binding partners are essential for HIV-1 reverse transcription *in vitro*. Moreover we have demonstrated that these translation factors can interact with components of the viral reverse transcription complex – RT and IN. siRNAs have been used to successfully down regulate the expression of two candidate proteins in 293T cells. The effect of this down regulation will be investigated during HIV infection.

Discussion: A reverse transcription enhancing activity has been purified from human cell lysate and candidate proteins identified by mass spectrometry. Selective depletion of two candidate factors has resulted in significant decreases in reverse transcription efficiency *in vitro*. Furthermore, these proteins were found to interact with components of the RTC. These results suggest that these 2 factors and/or their binding partners are essential for HIV-1 reverse transcription. Further investigation is required to verify that these host factors are relevant to HIV-1 replication *in vivo*. This research will both expand the current understanding of host-pathogen interactions and potentially elucidate novel targets for antiretroviral therapies.